

Nootropic effects of synthetic flavonoid derivatives on scopolamine induced memory impairment in mice via cholinesterase inhibition and antioxidant system

Mehreen Ghias^{1*}, Mohammad Shoaib¹, Syed Wadood Ali Shah¹,
Muhammad Naveed Umar², Shakir Ullah¹, Niaz Ali³, Ismail Shah⁴ and Shafiullah¹

¹Department of Pharmacy, University of Malakand, Khyber Pakhtunkhwa, Pakistan

²Department of Chemistry, University of Malakand, Khyber Pakhtunkhwa, Pakistan

³Department of Pharmacology, Institute of Basic Medical Sciences, Khyber Medical University, Khyber Pakhtunkhwa, Pakistan

⁴Department of Pharmacy, Abdulwali Khan University, Khyber Pakhtunkhwa, Pakistan

Abstract: The synthesized flavonoid derivatives (flavonols and flavones) were subjected for *in-vitro* anticholinesterase evaluation followed by assessment of *in-vivo* memory enhancing effects using animal models. The *ex-vivo* analysis of brain was carried out and portions were subjected for estimation of biochemical parameters that includes AChE, ACh, SOD and CAT level. Among tested flavonoids, the para substituted chloro containing flavonol (OF2) and flavone (F2) revealed a considerable *in-vitro* AChE and BuChE % inhibition with an IC₅₀ values. It was observed from the *in-vivo* results that OF1-OF3 at 12.5 mg/kg b.w has significance over F1-F3 in ameliorating the memory in scopolamine induced amnesic mice in passive avoidance step through and novel object recognitions test. Scopolamine elevated significantly the AChE level, decreased the contents of ACh, SOD and CAT in the brain in amnesic model. The flavonoid derivatives showed significant effects on these changes by decreasing the *ex-vivo* AChE contents, enhancing the level of ACh, SOD and CAT suggesting their possible role as cholinesterase and antioxidant. These findings suggest that synthetic flavonols and flavones may serve as potential candidates for developing safer and effective nootropic agents.

Keywords: Flavonols and flavones, memory, nootropics, *in-vivo* and *ex-vivo*, biomarkers.

INTRODUCTION

Learning is the process of acquisition of information and skills and its subsequent retention is called memory. Learning and memory is collectively termed as cognition (Bakoyiannis *et al.*, 2019) and is the most intensively explored discipline in the field of neuroscience (Ren, 2019). Memory is an elementary mental process by which impressions, sensations and ideas are stored and recalled. Cognitive dysfunction is an important feature of neurodegenerative disorder which is basic character of Alzheimer's disease (Ozarowski *et al.*, 2016). It starts with decline in short term memory, forgetting the addresses and names of individual. As this situation progresses, it becomes more worsen and even the person unable to find the way of his/her home (Javid and Rabiei, 2014). The centrally acting cholinergic system plays a major role in the process of memory by maintaining the normal level of acetylcholine (ACh) (Ferreira-Vieira *et al.*, 2016). In cognitive *impairment*, low level of ACh is observed that can be overcome by use of cholinesterase inhibitors (Lian *et al.*, 2017). Synthetic organic chemistry can contribute to the *discovery* of biologically active small molecules in several ways with neuropharmacological potentials (Danta and Piplani, 2016). Several studies have reported the use of plant extracts, their isolated compounds, semisynthetic natural products

and synthetic drugs as anticholinesterase inhibitors (Costanzo *et al.*, 2016; Malik *et al.*, 2016). Certain reports have claimed that synthetic compounds can act on the central nervous system, thereby enhancing the ability of learning and memory.

Synthetic and naturally occurring flavonoid derivatives have many remarkable pharmacological activities including their anticholinesterase potentials (Wang *et al.*, 2018). Due to the significance of flavonoids reported by our group and elsewhere (Ravishankar *et al.*, 2018; Shoaib *et al.*, 2019a; Shoaib *et al.*, 2019b; Shoaib *et al.*, 2016; Wang *et al.*, 2018), an effort was made to explore the possible role of antioxidant enzyme and cholinesterase potentials of synthesized flavonol and flavone derivatives as nootropics (memory enhancer) along with portraying the structure activity relationship (SAR).

MATERIALS AND METHODS

A series of flavonoid derivatives (OF1-OF3 and F1-F3) were prepared our group and the reaction conditions, yield along with spectroscopic data are reported (Shoaib *et al.*, 2019a, Shoaib *et al.*, 2016). 5,5-dithio-bis-nitrobenzoic acid (DTNB), Enzyme AChE *Electric eel*, BuChE equine serum Lyophilized, acetyl and butyrylthiocholine iodide, galanthamine hydrobromide, donepezil, Tween-80 were purchased from Sigma Aldrich, Germany. Mice Balb/C (18-23g) were purchased from the

*Corresponding author: e-mail: mehreenghias@yahoo.com

National Institute of Health (NIH) and kept in animal house with free access to food and *water ad libitum*. The animals were kept at room temperature around 22-25°C with light and dark cycle of about 12 h each (light on 6:00 am) and a relative humidity of 50-55%. Study was conducted as per approval from the Departmental Animal Ethical Committee vide notification no: NSF03/2017-122, in accordance with the Animals Byelaws 2008 of University of Malakand (Scientific Procedures Issue-I)".

In-vitro anticholinesterase activity

For the determination of acetyl cholinesterase inhibitory potentials, the AChE enzyme, solutions of DTNB and substrate ATChI against flavonol and flavone derivatives at various concentrations (Shoaib *et al.*, 2015a) were tested. In similar fashion, the butyrylcholinesterase inhibitory potentials of flavonol and flavone derivatives was assessed using BuChE enzyme, DTNB and BTChI and the absorbance was taken at 412 nm. Galanthamine and donepezil was used as positive control. The data was taken as triplicate and IC₅₀ was calculated (Shoaib *et al.*, 2015b).

Acute toxicity study

OF1-OF3 and F1-F3 were screened for its possible toxicological effects using Balb/C mice as model in two phases at different dose concentration ranging from 10 mg/kg to 500 mg/kg b.w by intraperitoneal (*i.p.*) route. Mice were observed for abnormal behaviors, allergic manifestation and mortality for the next 72 h followed by 14 days observation (Islam *et al.*, 2019).

Drug Treatment

OF1-OF3 and F1-F3 (12.5 mg/kg), Tween-80, donepezil (2 mg/kg) were administered via intraperitoneal route (*i.p.*) for 28 days to respective groups. Scopolamine at 1.5 mg/kg dose was intraperitoneally given to groups (control, test samples and donepezil) to produce amnesic effects. The apparatus used were cleaned prior to start of each experiment. The results were noted for acquisition phase and retention phase of memory for step through passive avoidance and novel object recognitions test.

Step through passive avoidance test

The test was performed in a chamber to evaluate the effects of OF1-OF3 and F1-F3 for memory enhancement of the mice. The chamber comprises of two compartments light and dark with grid floor facilitated by application of electric shock is used. Both the compartments are partitioned with guillotine door that facilitate the movement of mice easily. Study pattern consists of acquisition phase and retention phase. On the day of acquisition phase after acclimatization and habituation, mice in respective groups were placed to explore in the light compartment followed by opening of the guillotine door to permit entry to the dark chamber. Upon entrance, the door was shut down and an electrical foot shock (0.5

mA) was delivered to the grid floor for 3s. The mice were returned back immediately to its home cage. On the next day of acquisition phase (24 hrs later), mice were exposed to the retention phase to determine the memory in a similar manner like in acquisition devoid of applying foot shock. The step through latency (STL, time taken to enter into the dark compartment) was recorded using stopwatch for a maximum of 300s. Prior to acquisition trial, scopolamine (amnesic agent, 1.5mg/kg) was given 30 minutes before acquisition trial while synthesized flavonoids, donepezil and Tween-80 (vehicle) were given 60 minutes before the acquisition trial (de la Pena *et al.*, 2017; Lee *et al.*, 2018).

Novel object recognition test (NORT)

The NORT apparatus consist of a plexiglass box with modification (40 cm × 40 cm × 30 cm). The objects used for discrimination were made of plastic in two different shapes. After acclimatization, animals were allowed to walk around the empty box for habituation for 2-5 minutes one day before the test. On the day of test in the sample phase (T1), two identical objects were placed in two opposite corners of the apparatus and the time taken for exploration the objects was noted. Exploration was judged as mice directed its nose to the object at a distance less than 2cm and/or touching the object. During the test phase (T2) that was carried out 24 h after T1, a novel (new) object was replaced by one of the objects. The mice were placed in similar pattern as in T1 in the box and the time for exploration of the familiar (F) and new (N) object was noted separately. The discrimination index (DI) was calculated as $N-F/N+F$ (Pahaye *et al.*, 2017).

Assessment of biochemical parameters and biomarker study

The animals after nootropic behavioral study were decapitated under ether anesthesia and the brain from each animal was excised (fig. 1) to separate hippocampus and frontal cortex region in ice cold (chilled) phosphate buffer saline. The portions were subjected to analysis of biochemical parameters that include acetylcholinesterase (AChE), acetylcholine (ACh), superoxide dismutase (SOD) and catalase (CAT) (Mushtaq *et al.*, 2018).

STATISTICAL ANALYSIS

Data is taken as mean ± SEM. One-way ANOVA was used followed by Dunnett's multiple comparison tests for finding statistical significance using GraphPad prism 5 software version 5.01, San Diego, CA, USA. Effects were considered to be statistically significant at P value less than 0.05.

RESULTS

The structure of reported flavonol and flavone derivatives used in the memory enhancing study is given in fig. 2.



Fig. 1: Grouping, dosing and preparation for biomarker study

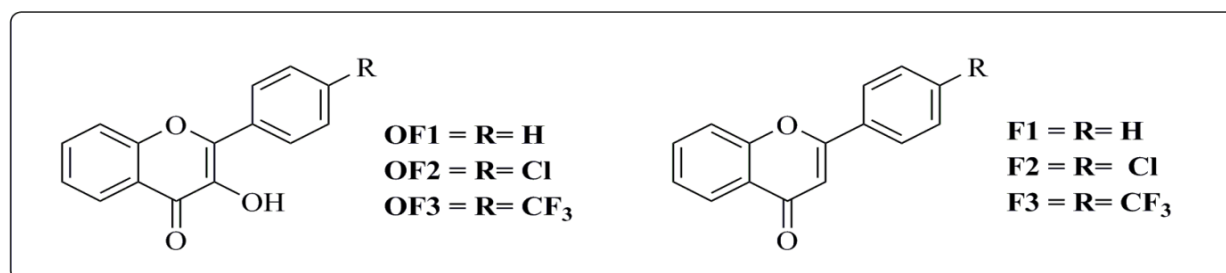


Fig. 2: Flavonoids used in study for memory enhancing effects

Table 1: Cholinesterase activity of synthetic flavonoids.

Sample Test	AChE IC ₅₀ (µg/mL)	BuChE IC ₅₀ (µg/mL)
OF1	129.11±1.39	188.59±1.06
OF2	98.61±2.01	152.34±1.41
OF3	121.18±1.79	185.86±1.88
F1	187.23±1.65	244.78±1.81
F2	113.65±1.06	166.35±1.52
F3	130.25±1.53	219.67±1.91
Galanthamine	12.81±1.02	13.69±1.70
Donepezil	5.19±1.31	7.39±1.62

Data are taken as mean ± SEM (n = 3).

Table 2: Nootropic effects in passive avoidance step through task for flavonoids

Sample Test	Dose (mg/kg b.w)	Acquisition (sec)	Retention (sec)
Control	Tween 80	38.30±4.25	188.14±4.59
Scopolamine	1.5	49.11±4.75	52.01±3.98 ^{†††}
Donepezil	2	32.87±5.54	206.33±3.51 ^{***}
OF1	12.5	51.56±3.25	106.31±3.22 [*]
OF2	12.5	51.31±3.89	111.91±4.11 ^{**}
OF3	12.5	38.33±2.78	95.41±4.88 [*]
F1	12.5	32.11±3.67	100.12±4.19 [*]
F2	12.5	45.05±3.52	107.19±3.19 [*]
F3	12.5	41.63±3.51	87.93±4.15 [*]

Mean ± SEM (n = 8). ^{†††}P<0.001 vs control normal group. ^{*}P<0.05, ^{**}P<0.01, ^{***}P<0.001 vs scopolamine-treated group (One-Way ANOVA followed by Dunnett's bilateral comparisons).

Table 3: Nootropic effects of synthetic flavonoids

Sample Test	Dose	SAMPLE		TEST		DI
		IO A1	IO A2	NO A1	FO A2	
Control	T-80	21.43±5.24	23.61±4.02	36.92±4.87	9.19±4.14	60.14±3.92
Scopolamine	1.5	11.01±4.04	12.09±2.19	16.26±3.92 ^{!!!}	9.01±5.09	28.69±4.02
Donepezil	2	19.99±4.24	19.84±3.24	42.52±3.69 ^{***}	8.41±2.47	66.98±5.11
OF1	12.5	14.15±3.84	15.11±5.11	35.71±4.11 [*]	9.91±2.90	59.34±2.98
OF2	12.5	16.44±4.21	17.24±3.56	41.35±3.78 ^{***}	9.14±5.71	63.77±4.02
OF3	12.5	13.06±2.98	14.09±3.67	33.15±4.67	9.61±5.19	55.05±2.19
F1	12.5	14.11±4.04	15.10±5.15	34.34±5.08 [*]	8.92±4.04	58.76±4.04
F2	12.5	14.32±5.15	15.66±4.02	36.91±2.98 [*]	9.41±5.15	59.36±2.56
F3	12.5	13.11±5.15	14.76±3.92	31.16±4.02	9.12±4.04	54.71±4.87

Mean ± SEM (n = 8). ^{!!!}P<0.001 vs control normal group. ^{*}P<0.05, ^{**}P<0.01, ^{***}P<0.001 vs scopolamine-treated group (One-Way ANOVA followed by Dunnett's bilateral comparisons).

Table 4: Effect of flavonoids on AChE and ACh level in brain portions

Sample Test	AChE (µmoles of substrate hydrolysed / min / g tissue)		ACh (mmol/min/mg protein)	
	Frontal Cortex	Hippocampus	Frontal Cortex	Hippocampus
Control	11.20±1.41	12.73±1.67	15.62±1.40	17.31±1.27
Scopolamine	25.76±0.79 ^{§§§}	30.62±1.15 ^{§§§}	5.93±1.48 ^{###}	6.54±1.15 ^{†††}
Donepezil	11.81±1.88 ^{***}	13.38±1.43 ^{***}	14.07±1.70 ^{***}	15.27±1.14 ^{***}
OF1	16.67±1.49 [*]	17.92±1.19 [*]	10.19±1.07 [*]	11.31±1.23 [*]
OF2	13.92±1.24 ^{***}	16.33±1.11 ^{**}	12.71±1.10 ^{**}	13.84±1.44 ^{**}
OF3	16.32±1.41 [*]	18.71±1.19 [*]	10.40±1.18 [*]	11.51±1.06 [*]
F1	17.64±1.24 ^{**}	18.83±1.55 ^{**}	9.67±1.27 ^{**}	11.11±1.17 ^{**}
F2	14.21±1.31 ^{**}	17.81±1.61 ^{***}	12.09±1.78 ^{**}	12.61±1.45 ^{**}
F3	18.67±1.45 ^{**}	18.91±1.61 ^{**}	9.07±1.54 ^{**}	10.19±1.57 ^{**}

Mean ± SEM (n = 8). ^{§§§}P<0.001, ^{§§§}P<0.001, ^{###}P<0.001 and ^{†††}P<0.001 vs control normal group. ^{*}P<0.05, ^{**}P<0.01, ^{***}P<0.001 vs scopolamine-treated group (One-Way ANOVA followed by Dunnett's bilateral comparisons).

Cholinesterase inhibitory activity

The summarized results in table 1 are presented for ChEI of the flavonols (OF1-OF3) and flavones (F1-F3). The simple flavonol and flavone (OF1 and F1) exhibited a % inhibition for AChE at concentration (62.5-1000 µg/ml) with an IC₅₀ value of 129.11±1.39 and 187.23±1.65 µg/ml respectively while IC₅₀ value of 188.59±1.06 and 244.78±1.81 µg/ml for OF1 and F2 respectively was observed for BuChE inhibition. The standard exhibited significant percent AChE and BuChE inhibition with IC₅₀ of 12.81±1.02 and 13.69±1.70 µg/ml respectively for standard galanthamine and IC₅₀ of 5.19±1.31 and 7.39±1.62 µg/ml respectively for standard donepezil. In contrast to other flavonoids, the dominant results were shown by OF2 and F2.

The para substituted chloro containing flavonol (OF2) and flavone (F2) revealed a considerable AChE % inhibition with an IC₅₀ value of 98.61±2.01 and 113.65±1.06 µg/ml respectively. Similarly in BuChE inhibition assay, IC₅₀ values of 152.34±1.41 and 166.35±1.52 µg/ml were observed for OF2 and F2.

Acute toxicity study

The synthesized flavonol and flavone derivatives were screened for its possible toxicological effects using mice

as model. There were no signs of abnormal behaviors, allergic reactions, mortality and were found to possess low toxicity profile. The compounds were found to be safe upto 500mg.

Passive avoidance task

Scopolamine administration resulted in the decline of memory and learning skills in amnesic mice showed deficits in response. The amnesic group (scopolamine induced) displayed decrease in the STL response to 52.01 sec (n=8) when compared to control that showed 188.14 sec (n=8). The administration of donepezil increased the STL response from 32.87 sec to 206.33 sec (P<0.001, n=8) and was significant when compared to group treated with scopolamine. The synthetic flavonoids (OF1-OF3 and F1-F3) treatment at a dose 12.5 mg/kg b.w. significantly ameliorated the memory and the findings are presented in the table 2.

It is understandable from the findings that OF1-OF3 at 12.5mg/kg b.w has significance over F1-F3 in ameliorating the memory. OF1-OF3 at the dose of 12.5 mg demonstrated rise in the STL of (106.31 sec, P<0.05), (111.91 sec, P<0.01) and (95.49 sec P<0.05) respectively as compared to amnesic group (52.01 sec) evidencing the improvement of effects produced by scopolamine. F1-F3

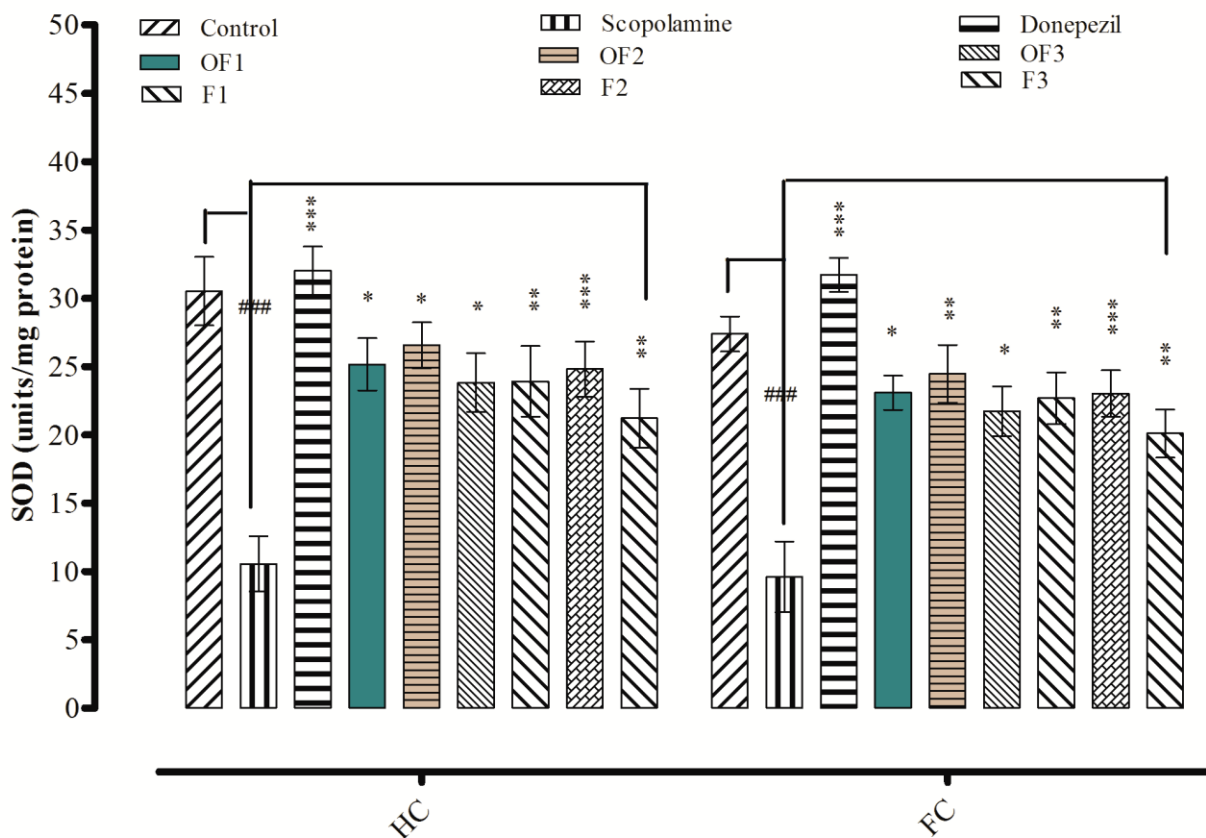


Fig. 3: SOD level of flavonoids in nootropic study. Mean \pm SEM ($n = 8$). ### $P < 0.001$ vs control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs scopolamine-treated group (One-Way ANOVA followed by Dunnett's bilateral comparisons).

at 12.5 mg/kg b.w. showed rise in the STL of (100.12 sec, $P < 0.05$), (107.19 sec, $P < 0.05$) and (87.93 sec $P < 0.05$) respectively.

Among the test compounds, the OF2 and F2 being having *halogen chlorine* group at para position showed increase in the STL in comparison to amnesic group indication the possible influence of this group in enhancement of the activity. On the other hand, presence of *trifluoromethyl* group (*electron-withdrawing*) at para position in OF3 and F3 decrease the latency response of the flavonoids indicating the incorporation *electron-withdrawing* group may cause reduction in nootropic effects. Overall, the synthetic flavonoids at 12.5mg/kg resulted in a significant rise in the STL response and upturned the amnesia induced scopolamine ($P < 0.05$, $P < 0.01$, $P < 0.001$).

Novel object recognition (NOR) Test

Results from nootropic behavioral study of flavonoid derivatives on NOR test model are shown in table 3. In sample phase, no change was observed significantly in exploration time for the objects for all samples tested groups. In the test phase, exploration time was significantly for novel object was noted than the identical object in groups treated with synthetic flavonoids at 12.5

mg/kg and donepezil at 2mg/kg along scopolamine at 1.5 mg/kg.

Exploration time for the novel object was increased significantly ($P < 0.001$, $n=8$) by donepezil decreased for familiar one with discrimination index of 66.98%. The lowest DI (28.69%, $^{***}P < 0.001$) was observed in group treated with scopolamine in comparison to control group.

Flavonols (OF1-OF3) significantly increased the discrimination index by 59.34%, 63.77% and 55.02% respectively at 12.25mg/kg b.w. respectively. Similarly for F1-F3, it was found to be 58.76%, 59.36% and 54.71% respectively.

Biochemical parameters and biomarker study

Scopolamine in this study significantly elevated the AChE level, decreased the content of ACh, decreases in the SOD and CAT level in the brain as an indicator of boosted oxidative stress in mice. The flavonoids showed significant effects on these changes by decreasing the AChE level, enhancing in the level of ACh, SOD and CAT content indicating the possible role of flavonoids as cholinesterase inhibitor and antioxidant in amnesic model.

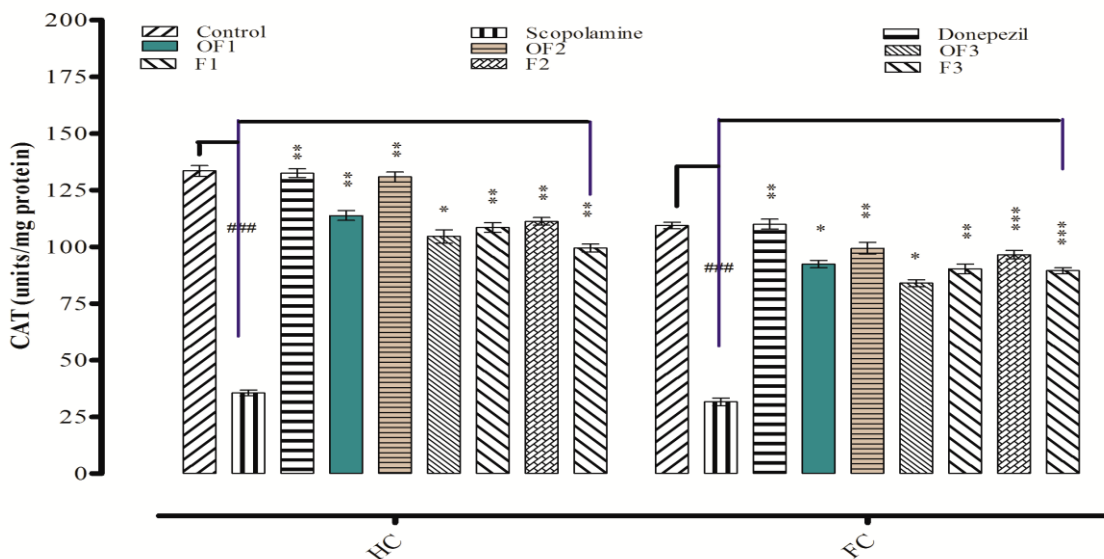


Fig. 4: Catalase level of flavonoids in nootropic study. Mean \pm SEM ($n = 8$). ### $P < 0.001$ compared to control normal group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ are compared to scopolamine-treated group (One-Way ANOVA followed by Dunnett's bilateral comparisons).

Effect on acetylcholinesterase and acetylcholine level

In this study, scopolamine substantially elevated the AChE level in the hippocampus and frontal cortex (table 4), that was efficiently reversed by the donepezil and tested flavonoids ($P < 0.05$, $P < 0.01$, $P < 0.001$, $n = 8$) suggesting its potentials to treat memory impairment possibly via ChE inhibitory effects. At the same time, a significant fall in the content of ACh was also recorded in amnesic group (table 4) that was reverted by flavonoids and standard as shown in the results.

To sum up, fall in the level of AChE enzyme was significantly observed and its role to hydrolyze the ACh was minimized that in turn significantly increased the level of ACh by synthetic flavonoids and standard donepezil confirming that the nootropic effects of flavonoids.

Effect on superoxide dismutase (SOD) and catalase (CAT) level

The administration of scopolamine resulted in significant decrease level of SOD by 10.56 ± 2.02 units/mg protein, $P < 0.001$, $n = 8$ in HC region and 9.61 ± 2.59 units/mg protein, $P < 0.001$, $n = 8$ of SOD level in FC region of the brain compared to control (30.51 ± 2.51 units/mg protein, $n = 8$) (fig. 3). The level was decrease by 2.89 in HC and 2.85 folds in the FC region of the brain in the amnesic group. This decrease in the level was overturned by the mice pretreated with donepezil to 3.04 folds and the level was recorded to 32.09 ± 1.78 units/mg protein, $P < 0.001$, $n = 8$) in the hippocampus. While in frontal cortex it was found to be 3.30 folds to a level of 31.72 ± 1.25 units/mg protein, $P < 0.001$, $n = 8$) when compare to scopolamine treated group. Pretreatment of mice with synthetic

flavonoids produced significant increase in SOD level in the brain regions.

Treatment of OF1-OF3 significantly increased SOD level in the hippocampus by 25.16 ± 1.91 units/mg protein, $P < 0.05$, $n = 8$), 26.56 ± 1.66 units/mg protein, $P < 0.05$, $n = 8$) and 23.83 ± 2.15 units/mg protein, $P < 0.05$, $n = 8$) compared with amnesic (scopolamine) group. Similarly significant increase in the SOD level was noted in frontal cortex for OF1-OF3. The flavone derivatives F1-F3 also increased SOD level in the brain to a significant level and is comparable to scopolamine treated group.

One way analysis of variance (ANOVA) produced the considerable output of synthetic flavonoid derivatives on the level of catalase in brain regions. In comparison with control (133.50 ± 2.33 , fig. 4), scopolamine administration caused significant fall of catalase level 35.58 ± 1.29 , $P < 0.001$, ($\downarrow 3.37$ folds) and 31.60 ± 1.67 , $P < 0.001$ ($\downarrow 3.37$ folds) in hippocampus (HC) and frontal cortex (FC) respectively. Donepezil significantly increased the level to 132.52 ± 1.91 , $P < 0.001$ ($\uparrow 3.72$ folds) and 110.02 ± 2.17 ($\uparrow 3.48$ folds,) in the brain regions.

Pretreatment with synthetic flavonoids (OF1-OF3 and F1-F3) produced similar response to standard and significantly increases the level of catalase in comparison to amnesic group. Scopolamine in this study significantly boosted oxidative stress in mice as indicated from the decrease level of SOD and CAT. The flavonoids produced significant effects on these alterations by enhancing in level SOD and CAT content indicating the possible role of flavonoids on oxidative stress as antioxidant agent.

DISCUSSION

It has been reported that acetylcholine (ACh) plays an imperative role in cognitive functions, mainly memory and learning (Maurer and Williams, 2017). A number of experimental reports have highlighted the correlation of ACh with learning and memory impairments in different type of animal models for assessment of memory enhancers (Garcia and Esquivel, 2018) and dysfunction of cholinergic system is taught to be responsible for AD symptoms (Boudouda *et al.*, 2015). At present, the most reliable approach for treating AD is cholinesterase inhibitors to enhance level of acetylcholine in the brain that include donepezil, tacrine, rivastigmine and galantamine (Girek and Szymanski, 2019). Passive avoidance task and exploration of novelty has been frequently employed in behavioral studies related to neuroscience and used as a tool for assessment of memory (Garcia and Esquivel, 2018).

In the brain, cholinesterase enzyme breaks apart the neurotransmitter acetylcholine, which is vital for the transmission of nerve impulses. The use of cholinesterase inhibitors impedes the normal enzymatic breakdown of the little acetylcholine that is present and is one of the treatment approaches for AD. That's why these problems can be trounced by achieving the level of neurotransmitter adequately to restrain cholinesterase by using agents called as anticholinesterase. Both superoxide dismutase and catalase antioxidant enzymes play a pivotal role in the defense against oxidative stress (Younus, 2018) that fights against reactive oxygen species to minimize the risk of neurodegenerative disorders.

Phenolic compounds like flavonoids have been reported as multi potent substances in fighting Alzheimer's disease by enhancing levels of acetylcholine (Khan *et al.*, 2018). Acetyl cholinesterase inhibitors may have a possible memory enhancing role in the management AD (Akram and Nawaz, 2017). Flavonoids from species of salvia are reported to possess cholinesterase inhibition potentials on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) (Lopresti, 2017). It has also has been suggested that kaempferol, galangin, myricetin and other flavonoids could significantly improve learning capability via cholinergic and antioxidant system (Flanagan *et al.*, 2018; Wang *et al.*, 2018).

Due to the significance of flavonoids reported by our group and elsewhere (Ravishankar *et al.*, 2018; Shoaib *et al.*, 2019a; Shoaib *et al.*, 2019b; Shoaib *et al.*, 2016; Wang *et al.*, 2018), an effort was made to explore the possible role of antioxidant enzyme and cholinesterase potentials of synthesize the flavonol and flavone derivatives as nootropics.

CONCLUSION

In conclusion, the present investigation portray that synthetic flavonol and flavone derivatives have significant *in-vitro*, *in-vivo* and *ex-vivo* memory enhancing effects using scopolamine induced amnesic model. The findings suggest that synthetic flavonoid derivatives can be used as a safer and effective candidate for development of nootropic agents.

REFERENCES

- Akram M and Nawaz A (2017). Effects of medicinal plants on Alzheimer's disease and memory deficits. *Neura. Regen. Res.*, **12**(4): 660-670.
- Bakoyiannis I, Daskalopoulou A, Pergialiotis V and Perrea D (2019). Phytochemicals and cognitive health: Are flavonoids doing the trick? *Biomed Pharmacother.*, **109**(1): 1488-1497.
- Boudouda, HB, Zeghib A, Karioti A, Bilia AR, Ozturk M, Aouni M, Kabouche A and Kabouche Z (2015). Antibacterial, antioxidant, anti-cholinesterase potential and flavonol glycosides of *Biscutella raphanifolia* (Brassicaceae). *Pak. J. Pharm. Sci.*, **28**(1): 153-158.
- Costanzo P, Cariati L, Desiderio D, Sgammato R, Lamberti A, Arcone R, Salerno R, Nardi M, Masullo M and Oliverio M (2016). Design, Synthesis, and Evaluation of Donepezil-Like Compounds as AChE and BACE-1 Inhibitors. *ACS Med. Chem. Lett.*, **7**(5): 470-75
- Danta C and Piplani P (2017). Design, synthesis and molecular docking studies of new potential piperazine derivatives as cognition enhancers. *Cent. Nerv. Syst. Agents Med Chem.*, **17**(2): 157-170.
- dela Pena IJI, Kim HJ, Botanas CJ, de la Pena JB, Van Le TH, Nguyen MD, Park JH and Cheong JH (2017). The psychopharmacological activities of Vietnamese ginseng in mice: Characterization of its psychomotor, sedative hypnotic, antistress, anxiolytic and cognitive effects. *J Ginseng Res.*, **41**(2): 201-08.
- Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM (2016). Alzheimer's disease: Targeting the Cholinergic System. *Curr neuropharmacol.*, **14**(1): 101-15
- Flanagan E, Muller M, Hornberger M and Vauzour D (2018). Impact of Flavonoids on Cellular and Molecular Mechanisms Underlying Age-Related Cognitive Decline and Neurodegeneration. *Curr. Nutr. Rep.*, **7**(2): 49-57.
- Garcia Y and Esquivel N (2018). Comparison of the Response of Male BALB/c and C57BL/6 Mice in Behavioral Tasks to Evaluate Cognitive Function. *Behav. Sci.*, **8**(1): 14-22.
- Girek M and Szymanski P (2019). Tacrine hybrids as multi-target-directed ligands in Alzheimer's disease: influence of chemical structures on biological activities. *Chem. Pap.*, **73**(2): 269-289.

- Islam S, Shajib MS, Rashid RB, Khan MF, Al-Mansur MA, Datta BK and Rashid MA (2019). Antinociceptive activities of *Artocarpus lacucha* Buch-ham (Moraceae) and its isolated phenolic compound, catechin, in mice. *BMC Complement Altern Med.*, **19**(1): 214-26
- Jivad N and Rabiei Z (2014). A review study on medicinal plants used in the treatment of learning and memory impairments. *Asian. Pac. J. Trop Biomed.*, **4**(10): 780-89
- Khan H, Marya, Amin S, Kamal MA and Patel S (2018). Flavonoids as acetylcholinesterase inhibitors: Current therapeutic standing and future prospects. *Biomed Pharmacother.*, **101**(1): 860-870.
- Lee JE, Song HS, Park MN, Kim SH, Shim B-S and Kim B (2018). Ethanol Extract of *Oldenlandia diffusa* Herba Attenuates Scopolamine-Induced Cognitive Impairments in Mice via Activation of BDNF, P-CREB and Inhibition of Acetylcholinesterase. *Int. J. Mol. Sci.*, **19**(2): 363-375.
- Lian W, Fang J, Xu L, Zhou W, Kang D, Xiong W, Jia H, Liu AL and Du GH (2017). DL0410 ameliorates memory and cognitive impairments induced by scopolamine via increasing cholinergic neurotransmission in mice. *Molecules.*, **22**(3): 410-423.
- Lopresti, AL (2017). Salvia (Sage): A Review of its Potential Cognitive-Enhancing and Protective Effects. *Drugs in R & D.*, **17**(1): 53-64.
- Malik R, Gupta R, Srivastava S, Choudhary BS and Sharma M (2017). Design, synthesis and biological evaluation of selected 3-[3-(amino) propoxy] benzenamines as acetylcholinesterase inhibitors. *J. Biomol. Struct. Dyn.*, **35**(11): 2382-2394.
- Maurer SV and Williams CL (2017). The cholinergic system modulates memory and hippocampal plasticity via its interactions with non-neuronal cells. *Front Immunol.*, **8**(1): 1489-1503.
- Mushtaq A, Anwar R and Ahmad M (2018). Lavandula stoechas (L) a Very Potent Antioxidant Attenuates Dementia in Scopolamine Induced Memory Deficit Mice. *Front Pharmacol.*, **9**(1): 1375-1385.
- Ozarowski M, Mikolajczak PL, Piasecka A, Kachlicki P, Kujawski R, Bogacz A, Bartkowiak-Wieczorek J, Szulc M, Kaminska E and Kujawska M (2016). Influence of the *Melissa officinalis* Leaf Extract on Long-Term Memory in Scopolamine Animal Model with Assessment of Mechanism of Action. *Evid Based Complement Alternat Med.*, **2016**(1): 1-17.
- Pahaye DB, Bum EN, Taiwé GS, Ngoupaye GT, Sidiki N, Moto FCO, Kouemou N, Njapdounke SJK, Nkantchoua G and Kandeda A (2017). Neuroprotective and anti-amnesic effects of *Mitragyna inermis* willd (Rubiaceae) on scopolamine-induced memory impairment in mice. *Behav. Neurol.*, **2017**(1): 1-11.
- Ravishankar D, Salamah M, Akimbaev A, Williams HF, Albadawi DAI, Vaiyapuri D, Greco F, Osborn HMI and Viayapuri S (2018). Impact of specific functional groups in flavonoids on the modulation of platelet activation. *Sci. Rep.*, **8**(1): 9528-9536.
- Ren F (2019). Influence of Cognitive Neuroscience on Contemporary Philosophy of Science. *Transl Neurosci.*, **10**(1): 37-43.
- Shoaib M, Shah SWA, Ali N, Shah I, Naveed UM, Ayaz M, Tahir MN and Akhtar S (2015a). *In vitro* enzyme inhibition potentials and antioxidant activity of synthetic flavone derivatives. *J. Chem.*, **2015**(1): 1-7.
- Shoaib M, Shah I, Ali N and Shah SWA (2015b). *In vitro* acetylcholinesterase and butyrylcholinesterase inhibitory potentials of essential oil of *Artemisia macrocephala*. *Bangladesh J. Pharmacol.*, **10**(1): 87-91.
- Shoaib M, Shah S, Ali N, Shah I, Umar MN, Shafiullah, Tahir M and Ghias M (2016). Synthetic flavone derivatives. An antibacterial evaluation and structure-activity relationship study. *Bulg. Chem. Commun.*, **48**(3): 414-421.
- Shoaib M, Ghias M, Shah SWA, Ali N, Umar MN, Rahman M and Shah I (2019a). Synthetic flavonols and flavones: A future perspective as anticancer agents. *Pak J. Pharm. Sci.*, **32**(3): 1081-1089.
- Shoaib M, Shah SWA, Ali N, Umar N, Shah I and Tahir MN (2019b). A possible mechanistic approach of synthetic flavonoids in the management of pain. *Pak. J. Pharm. Sci.*, **32**(3): 911-917.
- Wang TY, Li Q and Bi KS (2018). Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. *Asian J. Pharm Sci.*, **13**(1): 12-23.
- Younus H (2018). Therapeutic potentials of superoxide dismutase. *Int. J. Health Sci., (Qassim)* **12**(3): 88-93.